Utilization of Freeze-killed House Fly and Stable Fly (Diptera: Muscidae) Pupae by Three Pteromalid Wasps[1]

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ABSTRACT Utilization of freeze-killed house fly, *Musca domestica* L., and stable fly, *Stomoxys calcitrans* L., pupae for development by the pteromalid parasitoids, *Muscidifurax zaraptor* Kogan and Legner, *Pachycrepoideus vindemiae* (Rondani) and *Spalangia nigroaenea* Curtis was investigated. All three species of parasitoids used both fly species as hosts. Parasitoid emergence from house fly and stable fly puparia was not significantly different for *M. zaraptor* and *P. vindemiae*. However, emergence of *S. nigroaenea* was significantly higher from house fly puparia than from stable fly puparia. When given a choice, all three pteromalid species preferred ovipositing on house fly pupae, as indicated by parasitoid emergence. Parasitoid emergence from house fly puparia comprised ca. 75, 58 and 90% of the total combined emergence from house fly and stable fly puparia for *M. zaraptor, P. vindemiae and S. nigroaenea,* respectively. For *M. zaraptor*, the proportion of female progeny from house fly puparia (ca. 60%) was significantly higher than that from stable fly puparia (ca. 47%). The proportion of females from house fly and stable fly puparia for *P. vindemiae* and *S. nigroaenea* was not significantly different.

KEY WORDS Insecta, pteromalid wasps, filth flies, host utilization, *Muscidifurax zaraptor, Pachycrepoideus vindemiae, Spalangia nigroaenea, Musca domestica, Stomoxys calcitrans.*

Surveys of filth fly parasitoids from throughout the world indicate the most commonly encountered species belong to the genera *Muscidifurax* and *Spalangia* (Legner and Olton 1968, Legner et al. 1967, Ables and Shepard 1976). Although surveys provide meaningful information, incomplete host records may occur because of systematic revisions and/or advancements. For example, the genus *Muscidifurax* was revised in 1970 and split from a monotypical genus into one containing four species (Kogan and Legner 1970). As a result, surveys conducted prior to 1970 lumped all known hosts to the one species within the genus *Muscidifurax.* Since the revision, difficulty in knowing key characteristics for species identification has created problems with data collection and establishing accurate host records.

In Nebraska, *Spalangia nigroaenea* Curtis and *Muscidifurax zaraptor* Kogan and Legner are the most frequently encountered filth fly parasitoids (Petersen and Meyer 1983a); *Pachycrepoideus vindemiae* (Rondani) is found occasionally.

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These species are capable of surviving the harsh winter climate of the midwest in hosts within fly breeding habitats (Guzman and Petersen 1986, Petersen and Meyer 1983b, Petersen and Pawson 1988). Because these wasps emerge in the spring when numbers of available hosts are low, studies have been conducted to determine the suitability of using freeze-killed hosts for wasp propagation. Several studies indicate that these species will develop on freeze-killed house fly pupae (Petersen and Matthews 1984, Pickens and Miller 1978). The purpose of this study was to determine if freeze-killed stable fly (*Stomoxys calcitrans* L.) pupae are suitable hosts for *M. zaraptor, P. vindemiae* and *S. nigroaenea*, and if given a choice, would they prefer freeze-killed house fly (*Musca domestica* L.) or stable fly pupae for oviposition/development.

Materials and Methods

House fly and stable fly pupae and parasitoids used in this study were obtained from colonies maintained at the USDA Midwest Livestock Insects Research Unit at the University of Nebraska. The house fly and stable fly colonies were established from flies collected from feedlots and dairies in eastern Nebraska and have been periodically supplemented with wild flies to maintain colony vigor. During this study, length of house fly pupae ranged from 6.0 to 7.0 mm and length of stable fly pupae ranged from 5.0 to 6.0 mm. The colonies of *M. zaraptor* and *S. nigroaenea* were established from parasitized house fly pupae collected at dairies in eastern Nebraska in 1986 and 1987, respectively. The colony of *P. vindemiae* was initially supplied by E. F. Legner, University of California, Riverside, in 1984. When the experiment was initiated, colonies of *M. zaraptor, P. vindemiae* and *S. nigroaenea* had been reared in the laboratory for ca. 30, 60 and 15 generations, respectively.

Because house flies and stable flies were reared on different media, all pupae were rinsed in lukewarm water (25°C) to reduce odors from rearing media and then allowed to air dry. These pupae were freeze-killed at -10°C when 24 - 48 h old. All pupae were used within one week of being freeze-killed. At the start of the experiment, parasitoids (which had been reared on freeze-killed house fly pupae) were 24 - 48 h old and mated, but had no prior ovipositional experience.

Experimental Design. A 3 by 3 factorial, randomized complete block design was used. The three factors investigated were parasitoid species, host species and parasitoid density. The three parasitoid species tested were *M. zaraptor, P. vindemiae* and *S. nigroaenea.* The three host treatments were all house fly pupae (100 hosts), all stable fly pupae (100 hosts) and equal numbers of house fly and stable fly pupae (50 of each fly host). The three parasitoid densities were 2, 4 and 5 females per 100 hosts for *M. zaraptor* and *P. vindemiae,* and 4, 5 and 10 females per 100 hosts for *S. nigroaenea*. These parasitoid to host density levels were chosen to ensure that females were provided a surplus of hosts for determining ovipositional preference. The density levels for *S. nigroaenea* were higher than those used for *M. zaraptor* and *P. vindemiae* because previous studies have shown that *S. nigroaenea* produces very few progeny at lower density levels (Pawson and Petersen 1990). Each treatment combination (27 in all) was replicated 12 times (six replications per block).

Experimental protocol. The experimental procedure consisted of placing 100 freeze-killed house fly or 100 freeze-killed stable fly pupae in petri dishes (10-cm diam by 1.5 cm), or placing two groups of 25 freeze-killed house fly and two groups of 25 freeze-killed stable fly pupae in four-sectioned (10-cm diam by 1.5 cm) petri dishes. The groups of 25 house fly or 25 stable fly pupae were placed in opposing quarters of the four-sectioned petri dishes. After the freeze-killed pupae thawed at room temperature (21 ± 2°C) for 2 h, the appropriate number of females of a particular species was added to the petri dishes. Then, the petri dishes were placed in a rearing chamber maintained at 25°C, 55 ± 5% RH and 24 h DD for 24 h. After the parasitoids were removed, the fly pupae in the four-sectioned petri dishes were combined by species and placed in unsectioned petri dishes (5.5-cm diam by 1.5 cm). Petri dishes containing pupae exposed to *M. zaraptor* and *P. vindemiae* were held at 25°C, and petri dishes containing pupae exposed to *S. nigroaenea* were held at 30°C for parasitoid emergence. After emergence ceased, puparia that failed to produce parasitoid progeny were dissected to determine if any progeny died prior to emergence.

Experimental Analysis. Variance equality was tested among the nine treatments (three host treatments by three parasitoid densities) for each species using PROC.UNIVARIATE (SAS Institute 1985), and in all cases variances were unequal. When tested, parametric transformations were also unequal. As a result, data were analyzed following non-parametric rank transformations. All tests of significance were made on transformed data at the 0.05 alpha level. Tests between host treatments were made using the LSMeans procedure (SAS Institute 1985). A *t*-test was used to determine if each parasitoid species would utilize a particular host if given a choice; comparisons were made between the results obtained from house fly pupae with those obtained from stable fly pupae when both hosts were exposed simultaneously to the various parasitoid densities. Values reported in the text represent means of the raw data.

Results

Muscidifurax zaraptor. Females of *M. zaraptor* were capable of using freeze-killed house fly and stable fly pupae as hosts (Table 1). Total emergence levels of *M. zaraptor* from house fly, stable fly, and house fly and stable fly pupae (when both hosts were exposed simultaneously) were not significantly different at any density level. Increasing parasitoid density significantly increased parasitoid emergence from all host treatments. When both hosts were exposed simultaneously, emergence of *M. zaraptor* was significantly higher from house fly puparia than from stable fly puparia at all density levels; emergence from house fly puparia comprised ca. 75% of the total combined emergence from both hosts.

The number of female progeny produced by *M. zaraptor* was consistently influenced by host species (Table 1). At the two highest densities the number of female progeny that emerged was significantly higher from house fly puparia (ca. 59%) than from stable fly puparia (ca. 45%). When both hosts were exposed to *M. zaraptor* females, more females were produced on house fly pupae than on stable fly pupae at all density levels with the two lowest density levels (two and four females) being significantly different. Parasitoid density did not appear to influence the number of female progeny.

Table 1. Mean total and female emergence (%) of *M. zaraptor*, *P. vindemiae* and *S. nigroaenea* from house fly, stable fly, and house fly plus stable fly pupae after exposure to three parasitoid density levels

Response	Host treatment	Parasitoid density level‡ 2	4	5
	M. zaraptor			
Total emergence	100 HF*	18.5 a	32.3 a	40.9 a
	100 SF	17.4 a	26.2 a	32.4 a
	50 HF + 50 SF	20.9 a	32.2 a	42.3 a
	50 HF†	15.9*	25.0*	31.7*
	50 SF	5.0	7.2	10.6
Female emergence	100 HF*	63.9 a	60.3 a	57.2 a
	100 SF	48.7 a	47.7 b	43.4 b
	50 HF + 50 SF	54.2 a	53.2 a	54.2 a
	50 HF†	68.6*	62.8*	58.7
	50 SF	39.8	43.5	49.7
	P. vindemiae			
Total emergence	100 HF	30.7 a	30.7 a	55.3 a
	100 SF	41.0 a	52.5 a	56.6 a
	50 HF + 50 SF	40.0 a	53.9 a	61.4 a
	50 HF	22.5	32.9*	35.9*
	50 SF	17.5	21.0	25.5
Female emergence	100 HF	83.0 a	78.4 a	79.0 a
	100 SF	83.5 a	79.5 a	79.1 a
	50 HF + 50 SF	79.0 a	78.0 a	77.6 a
	50 HF	77.3	76.5	76.5
	50 SF	80.8	79.4	78.7
	S. nigroaenea			
Total emergence	100 HF	10.2 a	10.6 a	22.1 a
	100 SF	1.7 c	2.7 b	4.4 b
	50 HF + 50 SF	6.1 b	8.4 ab	9.3 b
	50 HF	5.8*	7.8*	8.9*
	50 SF	0.3	0.6	0.4
Female emergence	100 HF	55.2 a	68.6 a	60.3 a
	100 SF	45.0 a	41.7 b	53.6 a
	50 HF + 50 SF	58.3 a	64.6 a	62.4 a
	50 HF	52.0	52.6*	67.3
	50 SF	83.3	93.3	33.3

* House fly plus stable fly groups compared without respect to fly species. Values in the same column and within the same response followed by the different letters were significantly different, $P < 0.05$, LSMeans procedure.

† House fly plus stable fly groups compared with respect to fly species. Values in the same column and within the same response followed by an asterisk were significantly different, $P < 0.05$, t-test.

‡ Females per 100 hosts.

Pachycrepoideus vindemiae. As with *M. zaraptor, P. vindemiae* used both house fly and stable fly pupae for development (Table 1). Even though parasitoid emergence significantly increased as parasitoid density increased, there were no significant differences in emergence between the different host treatments. When exposed together, more *P. vindemiae* progeny were produced on house fly pupae than on stable fly pupae; significantly more progeny were produced at the two highest density levels (four and five females). The parasitoid emergence from house fly puparia accounted for 58% of the total combined emergence.

A high proportion of females (<75%) was produced on all host treatments parasitized by *P. vindemiae* females (Table 1). Neither parasitoid density nor host treatment had any effect on the number of female progeny.

Spalangia nigroaenea. Even though higher parasitoid densities were used, *S. nigroaenea* produced the lowest results of the three pteromalid wasps. Significantly more progeny were produced on house fly pupae than on stable fly pupae (Table 1). Even at higher density levels (ca. two times the levels were used for the other species), *S. nigroaenea* produced ca. 50% fewer progeny than *M. zaraptor* on freeze-killed house fly pupae. *Spalangia nigroaenea* appeared to prefer ovipositing in house fly rather than stable fly pupae as indicated by parasitoid emergence; emergence from house fly puparia was significantly higher than the emergence from stable fly puparia and made up more than 90% of the combined emergence at all density levels.

The emergence of *S. nigroaenea* females was similar to that of *M. zaraptor* (Table 1). Although the proportion of female progeny was lower on stable fly pupae (ca. 47%) than on house fly pupae (ca. 61%), only the proportion of females at the middle density (five females) was significantly different. The low number of females developing on stable fly pupae may have been caused by the low number of progeny that emerged from stable fly puparia. Because emergence was low when both hosts were exposed simultaneously to *S. nigroaenea,* the proportion of female progeny was highly variable. Both the highest and the lowest proportion of females were produced on stable fly pupae, but only the proportion of females on stable fly pupae at the middle density level (five females) was significant.

Discussion

All three species of parasitoids preferred to oviposit in freeze-killed house fly pupae when both house fly and stable fly pupae were exposed simultaneously, as indicated by parasitoid emergence. Whether this effect was a true "host" preference for each species or an artifact caused by rearing media odors, or because all three species were previously reared on house fly pupae is uncertain. Preference for a particular host would be an innate characteristic of a species; all three parasitoid species have been reared on house fly pupae for at least one year (ca. 8 to 10 generations), and whether "innate" preferences were established during this time is unknown. However, because *P. vindemiae* and *S. nigroaenea* have wide host ranges attacking at least 18 and 25 species of flies, respectively, including house flies and stable flies (Rueda and Axtell 1985), the possibility of establishing strong preferences for a specific host through rearing procedures so quickly is unlikely. The host range of *M. zaraptor* is not well known. Although they have

been separated taxonomically, researches still lump *M. raptor* and *M. zaraptor* together, producing inaccurate host records. Only one host, *M. domestica,* is listed for *M. zaraptor*. In any event, this study shows that both of these hosts can be used for development by these parasitoids.

The low parasitoid emergence levels for *S. nigroaenea* were not unexpected based on previous studies with similar results (Pawson and Petersen 1990). Although increasing the parasitoid density increased parasitism levels, parasitism levels for *S. nigroaenea* were still substantially below those for the other two species. Parasitoids have two distinctly different behaviors. They may be gregarious (a type of superparasitism) where many individuals develop on one host, or solitary where only one individual develops per host. Interestingly, the behavior of *S. nigroaenea* falls somewhere in the middle of these two extremes. If high parasitoid densities are exposed to house fly pupae, high parasitism rates can be obtained; whereas, at low parasitoid densities, extremely low parasitism rates are obtained (Pawson and Petersen 1990). More studies are needed to understand the factors influencing the behavior of *S. nigroaenea* and the factors that influence oviposition/parasitism.

Preference for live and freeze-killed house fly pupae has been studied for only *M. zaraptor* (Petersen et al. 1986). Discrimination appeared to occur at low parasitoid density levels, with *M. zaraptor* preferring to oviposit on live pupae; however, *M. zaraptor* readily used freeze-killed house fly pupae as hosts. Freeze-killed house fly pupae have been used to supplement releases of *M. zaraptor* to increase their population levels early in the season when fly populations are low (Petersen 1986). Whether *P. vindemiae* prefers to oviposit on live or freeze-killed house fly pupae needs to be evaluated to determine whether freeze-killed house fly pupae can be used to increase their populations early in the season. Present results indicate this procedure would not be advantageous for *S. nigroaenea* because low parasitoid emergence occurred even at high parasitoid densities.

The most studied factors affecting the proportion of female progeny of filth fly parasitoids are parasitoid and host densities. Generally, the proportion of female-to-male progeny increases as parasitoid densities decrease or host densities increase (Legner 1967, Pawson et al. 1987). Also, host size has been implicated in affecting the sex ratio of some parasitoids (Legner 1969) but not for others (Wylie 1967). In the study by Legner (1969) a giant, southwestern form of *M. raptor* was used. After taxonomic revision, it was identified as *M. zaraptor.* In the present study, only *M. zaraptor* showed significant differences in the sex ratio of their progeny. Further studies are needed to determine whether the sex ratio changes observed *M. zaraptor* can be attributed to host size or to different host species.

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